

DATA EVALUATION RECORD

Flutriafol
PC Code: 128940
TXR#: 0055512
MRID#: 48196922

Reproduction and Fertility Effects
OPPTS 870.3800
OECD 416

Flutriafol Technical:
Two-Generation Reproduction Study in the Han Wister Rat

Prepared for

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Flutriafof / PC Code 128940

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OPPTS 870.3800, OECD 416

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DATA EVALUATION RECORD**STUDY TYPE:** Reproduction and Fertility Effects – Flutriafof Technical: Two-Generation
Reproduction Study in the Han Wistar Rat (OPPTS 870.3800; OECD 416).**PC CODE:** 128940**DP BARCODE:** D383035**TXR #:** 0055512**TEST MATERIAL:** Flutriafof, 1-(2-Fluorophenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl)ethanol.**SYNONYMS:** Flutriafof [BSI:ISO]; (RS)-2,4'-Difluoro-alpha-(1H-1,2,4-triazol-1-ylmethyl)benzhydryl
alcohol; FLUTRIAFEN; Difenconazole.**CITATION:** Gerspach, R., Flade, D. and Chevalier, J. (2009). Flutriafof Technical: Two-
Generation Reproduction Study in the Han Wistar Rat. Harlan Laboratories Ltd.
(Former RCC Ltd.), Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland. Laboratories
Study Number A10563, MRID 481969-22.**Sponsor:** Cheminova A/S (EPA Company No. 67760), P.O.Box 9, DK-7620 Lemvig, Denmark.**EXECUTIVE SUMMARY**

In a two-generation reproduction toxicity study in rats (MRID # 481969-22), Flutriafof Technical (purity 95.1%, Batch no. UPL Bx 1 (2001) was administered at doses of 0, 30, 80, 150 or 300 ppm (0, 1.8/2.2, 4.8/5.85, 9/10.8, and 18.5/22.6 mg/kg bw/day [M/F] before and after pairing + gestation [F], 0, 4.45, 12.15, 22.5, and 46.2 mg/kg bw/day [F] during lactation) in the diet of Han Wistar rats (24 rats/sex/dose). P generation males and females were exposed to Flutriafof for 70 days prior to mating. Females continued to receive diet containing Flutriafof during the pairing, gestation and lactation periods. Males were also exposed after pairing until sacrifice. After weaning on post partum Day 21, F1 pups received Flutriafof at the same concentrations in their diet as their parents during their growth to adulthood, and during the pairing, gestation and lactation periods for breeding the F2 litters. All animals were checked twice daily for morbidity or mortality. All animals found dead were subjected to detailed macroscopic examination to establish cause of death, if possible. Animals were also checked twice daily for signs of reaction to treatment and/or symptoms of ill-health.

In P and F1 parental animals, no Flutriafof Technical-related clinical signs or observations were noted in this study. Mean and relative food consumption, body weights and body weight gains were also not affected by exposure to Flutriafof. The mean Flutriafof exposure dose levels achieved in the 300 ppm exposure group in this study were 20.8 mg/kg bw/day for P generation males, 22.1 mg/kg bw/day for F1 males, 23.9 mg/kg bw/day for P generation females and 24.5 mg/kg bw/day for the F1 generation females during the pre-pairing period. During gestation and lactation, P generation females in the 300 ppm group ingested 21.4 mg/kg bw/day and 44.9 mg/kg bw/day, respectively, while the F1 generation females in the 300 ppm group ingested 20.7 mg/kg bw/day and 47.5 mg/kg bw/day of Flutriafof. There were no Flutriafof-related abnormal findings in the F1 animals after weaning. Food consumption and relative food consumption were consistently non-statistically significant nor were they different from controls in a dose related manner. Small differences observed in body weight and body weight gain were also considered not related to Flutriafof treatment. Liver to body weight ratios were statistically significantly increased in both males and females in the 300 ppm group. There were no differences in any other organ weights or organ to body weight ratios between the control and Flutriafof exposed animals at any level of

exposure. There were no dose dependent abnormal macroscopic findings at necropsy that were Flutriafol related. Histopathology of livers showed slight increases in fatty deposits in both sexes and evidence of centrilobular hepatocellular hypertrophy in males in the 300 ppm group, which is considered an adaptive change.

The Parental LOAEL is 300 ppm (18.2/22.6 mg/kg bw/day [M/F]) based upon increased relative liver weights, fatty deposits in the liver in both sexes as well as hepatocellular hypertrophy in males. The NOAEL is 150 ppm (9/10.8 mg/kg bw/day[M/F]).

An increased incidence of pup mortality was observed in the F1 and F2 pups at first litter check and 0-4 postnatal days. Mean and relative organ weights were not affected by Flutriafol exposure. Abnormalities noted by macroscopic examination showed no dose response relationship with Flutriafol treatment levels. No pathological lesions were noted in the F2 pups.

The offspring LOAEL is 300 ppm (18.2/22.6 mg/kg bw/day [M/F]) based upon increased pup mortality in the F1 pups at the first litter check and 0-4 postnatal days. The offspring NOAEL is 150 ppm (9/10.8 mg/kg bw/day [M/F]).

There were no significant Flutriafol-related effects on the length of estrous cycle, mating performance and fertility in the P generation female rats. Sperm analysis data indicated no effects of Flutriafol on sperm motility or morphology at any of the doses used in this study. There were also no differences between the sperm head counts per gram of tissue in testes and caudal epididymus. The duration of gestation and the gestation index were also not affected by Flutriafol exposure. Breeding data for the P generation females show no statistically significant differences in the birth and viability indices. The weaning index for the 150 ppm group was statistically decreased compared to controls. Since the values in the 300 ppm group were not lower than controls, this difference was considered an incidental finding. There were also no statistically significant dose related effects in all other breeding indices. Sexual maturation in F1 females (days to vaginal patency) was not altered by Flutriafol treatment. In males, sexual maturation (days to preputial separation) showed a slight delay (statistically significant, yet within historical control data) in the 300 ppm compared to controls. There were no Flutriafol-related effects on estrous cycles and gestation index in the F1 animals. Fertility index and conception rates were not statistically significantly different between exposure groups and controls, however small, non-statistically significant decreases were evident in the highest (300 ppm) group. Duration of gestation, viability index and weaning index were not altered by Flutriafol treatment. Birth index for the 300 ppm was not statistically significantly different from controls, however it was lower than all the other groups. There were also no statistically significant differences in the breeding parameter values of the dose groups compared to controls, however the post-implantation loss measures were highest in the 300 ppm group as were the number of dead pups per litter. Mean organ weights and organ to body weight ratios were similar in F1 males and females in all the dose groups including controls and small changes were not related to Flutriafol dose. No Flutriafol-related effects were noted in sperm staging in males or differential follicle counts in females.

The reproductive LOAEL was not observed. The reproductive NOAEL is 300 ppm (18.2/22.6 mg/kg bw/day [M/F]).

This study is classified as **acceptable/guideline** for establishing a NOAEL for reproductive and fertility effects. It has met the requirements of the OPPTS 870.3800 and OECD 416 guidelines.

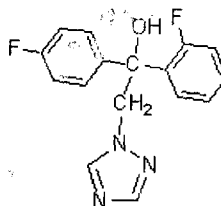
COMPLIANCE: Signed and dated Data Confidentiality, GLP Compliance, Flagging and Quality Assurance statements were provided.

Deficiencies: There were no deviations/deficiencies that impacted the scientific integrity of this study.

I. MATERIALS AND METHODS

A. Materials

1. **Test material:** Flutriafol Technical, Dry (CHA no. 131)
 - Description:** White solid
 - Lot/Batch#:** UPL Bx 1 (2001) (expiration date November 2008)
 - Purity:** 95.1%
 - CAS #:** 76674-21-0
 - Compound Stability:** Stable when stored at room temperature for at least 21 days, in the dark
 - Structure:**



2. **Vehicle:** Diet (Pelleted standard Kliba-Nafag 3433 Rat/Mouse Maintenance diet).

3. Test animals

- Species:** Rat,
- Strain:** HanRcc: WIST (SPF Quality)
- Age/ weight at study initiation:** 6 weeks, minimum
Males: Range 170-236 g
Females: Range 132-190 g.
- Source:** Harlan Laboratories Ltd, CH-4414 Füllinsdorf, Switzerland
- Housing:** Housed at Harlan Laboratories Ltd, Füllinsdorf under standard laboratory conditions individually in Makrolon cages (type-3) with wire mesh tops and standard granulated softwood bedding. During mating, animals were housed one male/one female in Makrolon pairing cages. After mating, males and females were housed individually again, males until necropsy and females for the birth and rearing of their litters.
- Diet:** Pelleted standard Kliba-Nafag 3433 available *ad libitum*
- Water:** Community tap water from Füllinsdorf in bottles available *ad libitum*.
- Environmental conditions:**
 - Temperature:** $22 \pm 3^{\circ}\text{C}$
 - Humidity:** 30 – 70%
 - Air changes:** 10-15 air changes per hour
 - Photoperiod:** 12 hr artificial fluorescent light/ 12 hr dark
- Acclimation period:** 7 days minimum under test conditions with an evaluation of the health status

4. Dose selection rationale:

Dose levels for the current study were selected on the basis of results from a preliminary reproductive toxicity study (RCC Study A52705) conducted in the rat with test substance (Flutriafol Technical) at doses of 0, 30, 60, 240, or 1000 ppm and recommendations of the OECD and OPPTS guidelines about the spacing factor between dosages. In preliminary study, a decrease in implantation number and increase in post implantation loss were reported at dose level of 1000 ppm. These effects were considered related to Flutriafol Technical exposure. Liver weights were

increased in both males and females, accompanied by fatty liver changes and hepatocellular hypertrophy. In addition, food consumption and body weight gains were also reduced. At dose level of 240 ppm, a slight increase in implantation loss, fatty liver changes in males (1000 for females) and hepatocellular hypertrophy in both sexes occurred. Based on these results a suitable top dose for the definitive study was determined to be 300 ppm.

5. Dose preparation and analysis:

Diet admixtures were prepared at least every 3 weeks by mixing test material with granulated diet. Water was added at a volume/weight ratio of 1:10 to ensure pelleting of the prepared diet. Pellets were dried for 48 hr prior to storage in closed stainless steel containers at room temperature. Samples for analyses of content of the test substance in the feed were drawn from every preparation, and analyzed. Homogeneity of the test item was determined before use of the first diet batches. For assessment of stability, samples were drawn from the middle of the dietary admixture on the day of preparation, stored at room temperature for 21 days and consequently frozen between -24 and -16 °C. Concentrations of the test substance in the diet at each dose were measured by GC-MS method. Dietary concentrations were used as supplied by the Sponsor and not corrected for purity of the test item.

Results

Analytical data in the study report indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

Homogeneity and concentration analysis: Diet samples were found to be homogeneously prepared. The concentration in the diet were found to be within the accepted range of $\pm 15\%$ of the nominal concentration. Recovery of Flutriafol in prepared rodent diet samples was found to be in the range of 75.0% to 114.9%.

Stability analysis: The test substance in the feed was stable for at least 21 days when stored at room temperature (based on the accepted variation limit of 10% from the time-zero concentration).

B. Study Design and Methods:

1. In Life Dates: Experimental starting date: November 27, 2007
Experimental completion date: March 31, 2009

2. Animal Assignment:

The study report states that animals were assigned randomly to test groups as shown in Table 1, however it also states that parent age and sibling relations were taken into consideration to ensure an even distribution across treatment groups. Also, body weights were taken into consideration to ensure similar mean body weights in all groups. P and F1 animals were identified by individual cage and ear number. Selection of F1 and F2 pups for different allocations (i.e. culling, necropsy on day 21 post partum, and rearing to breed the F2 generation) was based on random pup number on day 1 post partum. Each dose group contained 24 animals per sex for the Parental generation. Pups were individually tattooed with India ink. At the onset of hair growth, pups were identified by color spots on the fur. Rats in the four dose groups were given Flutriafol in their diet at the following concentrations: 0, 30, 80, 150 and 300 ppm. Control (Group 1) animals received the same diet but without the Flutriafol.

TABLE 1. Animal assignment					
Test group	Dose in diet (ppm)	Animals/group		Animals/litter	
		P Males	P Females	F ₁ Males ¹	F ₁ Females ¹

Group 1	0 ppm	24	24	24	24
Group 2	30 ppm	24	24	24	24
Group 3	80 ppm	24	24	24	24
Group 4	150 ppm	24	24	24	24
Group 5	300 ppm	24	24	24	24

On day 4 post partum for the F1 generation, the size of each litter was adjusted by eliminating extra pups to yield as near as possible 4 males and 4 females per litter. On day 21 post partum, 24 F1 males and 24 F1 females were selected for the F1 generation breeding studies.

3. Duration of Administration of Flutriafol in Diet

P Animals: Parental generation rats were exposed to the test diet during a pre-pairing period of 70 days, and also during the pairing period, gestation and lactation periods for breeding of the F1 litters.

F1 Animals: Following the weaning of the F1 litters on day 21 post partum, F1 animals were selected for the next generation. Treatment was started when the F1 animals were about four weeks of age but the animals were maintained on their diets from weaning. The test item was administered during growth of the F1 generation to adulthood (at least 90-day pre-pairing period) and also during the pairing, gestation and lactation periods for breeding the F2 litters.

4. Procedures, Observations and Data Recording

A) Procedures:

P Generation Breeding F1 Litters: Animals of the P generation were 7-8 weeks of age at the beginning of treatment. They were maintained on their respective test diets for 70 days prior to pairing. Vaginal smears were taken daily for 21 days prior to pairing and throughout the pairing period until the smear was sperm-positive or vaginal plug was observed. The animals were paired in the ratio of one male to one female for a maximum of 14 days. On day 4 post partum, pups were culled by random selection within each sex to give, as near as possible, 4 males and 4 females per litters. The dams were allowed to rear the remaining young to day 21 post partum. On or soon after day 21 post partum, 24 male and 24 female F1 pups per group were selected randomly from as many different litters as possible to provide the F1 generation. Excess pups were sacrificed and examined macroscopically. From all P animals (on day 21 post partum or shortly thereafter) and from one randomly selected male and female pup from each F1 litter (on day 21 postpartum precisely), selected organs and tissues were weighed and preserved.

F1 generation Breeding F2 Litters: The selected F1 animals were reared on their respective diets for at least 90 days prior to pairing. Vaginal smears were taken daily for 21 days prior to pairing and throughout the pairing period until the smear-positive or a vaginal plug was observed. The animals were paired in the ratio of one male to one female for a maximum of 14 days. The pairing of siblings was avoided. The dams were allowed to rear the remaining young to day 21 post partum. On or soon after day 21 post partum all F2 pups and F1 parent animals were sacrificed and examined histopathologically. From all F1 parent animals (on day 21 post partum or shortly thereafter) and from one randomly selected male and female pup of each F2 litter (on day 21 post partum precisely), selected organs and tissues were weighed and preserved.

B) Observations

Mortality Rate: All animals were checked twice daily for morbidity or mortality. All animals found dead were subjected to detailed macroscopic examination to establish cause of death, if possible.

Signs and/or Symptoms: Animals were also checked twice daily for signs of reaction to treatment and/or symptoms of ill health. Additionally, a thorough physical examination was conducted on each animals on weekly basis.

C) Data Recording

Body Weight: Animals were weighed on the first day of dosing and thereafter at weekly intervals, with the exception of the pairing period. After mating, females were weighed on days 0, 7, 14, and 21 post coitum. Dams which littered were weighed on days 1, 4, 7, 14 and 21 post partum and on the day the animals were sacrificed.

Food Consumption: Food consumption was measured weekly when body weights were recorded, except during the Pairing period. During Lactation, food consumption was recorded only until day 14 post partum.

Mating: A record of mating of females was made by daily examination of the vaginal smears for spermatozoa and/or appearance of a vaginal plug throughout the pairing period. The day on which evidence of mating was observed was considered to be day 0 post coitum. Once evidence of mating had been noted, the females were housed individually. The mating data were used to determine pre-coital time, detect whether or not pregnancy was interrupted after mating, detect marked anomalies of the estrous cycle, and determine duration of gestation. At necropsy, all uteri of parent females (P and F1 generation) were placed in a solution of ammonium sulfide to visualize possible hemorrhagic areas of implantation sites.

Gestation and Parturition: Towards the end of the gestation period, females were examined twice daily for signs of parturition. Females without litters were killed and necropsied together with the dams after weaning of the pups. Females which lost their litter were killed and necropsied directly after litter loss.

Lactation and Litter Data: Day 0 of lactation was the day on which a female had delivered all its pups. The litters were examined for litter size, live birth, stillbirth and any gross anomalies. Sex ratios of pups were recorded on day 0 and /or day 1, day 4, and day 21 of lactation. Litters were caged together with the dam until weaning on day 21 of lactation. Pups were weighed individually on day 0 and/or day 1, 4, 7, 14 and 21 of lactation. On day 1 post partum, pups were tattooed individually with India ink. At the onset of hair growth, the pups were identified by color spots on the fur. Dams and pups were observed daily for survival and behavioral abnormalities in nesting and nursing. Dead pups found until day 4 post partum, except those excessively cannibalized, were autopsied and preserved in ethyl alcohol (94%) for possible further examination.

Sexual Maturation: The age and body weight, at which vaginal opening or preputial separation occurred, was recorded for F1 generation weanlings selected for breeding the F2 generation.

5. Pathology

Termination and Postmortem Examination: All P and F1 adult animals selected for breeding were sacrificed when they were no longer necessary for the assessment of reproductive effects. Excess F1 and F2 pups after standardization of litter size were sacrificed on day 4 p.p, examined macroscopically and preserved in ethyl alcohol (94%). F1 pups not selected for the F1 generation pairing and all F2 pups were sacrificed, macroscopically examined, and fixed in neutral buffered 4% formalin after weaning. All parental generation animals (P and F1) were examined macroscopically for any structural abnormalities or pathological changes either at scheduled sacrifice or if death occurred during the study. Implantation sites were counted for all dams. The uteri were placed in a solution of ammonium sulfide to visualize possible hemorrhagic areas of implantation. Pups were macroscopically examined.

6. Organ Weights

The weights for the following organs were recorded from all P and F1 parent animals on day 21 post partum or shortly thereafter:

Uterus (including cervix, excluding oviducts) and Ovaries	Epididymides (total weight and cauda separately)	Seminal vesicles, with coagulating glands and fluids (as one unit)
Brain (including the. entire brainstem)	Kidney	Adrenal gland
Liver	Pituitary	Spleen
Thyroid	Testes	Prostate

Paired organs were weighed individually. The following organs were recorded from one randomly selected female pup of each F1 and F2 litter (on day 21 post partum precisely): brain, spleen and thymus.

7. Histopathology

Full histopathology was performed on the organs listed below for all high dose and control P and F1 animals selected for pairing, on all animals which died during the study (where practicable), and on one male and one female pup (selected for organ weight recording) of each F1 and F2 litter (high dose and control). At necropsy, samples of the collected tissues and organs were fixed in neutral phosphate buffered 4% formaldehyde solution. Organs demonstrating pathological changes in these animals were then examined in the animals from the dose groups. Microscopic examination of all tissues showing gross pathological changes was made. Microscopic examination of all reproductive organs (ovaries, uterus, cervix and vagina in females, epididymides, seminal vesicle and prostate for males) of all infertile animals, was made.

Gross lesions	Liver
Ovaries	Pituitary
Uterus and cervix	prostate
Adrenal glands	Vagina
Seminal vesicles with coagulating gland	Right testis and epididymis

Detailed Testicular Histopathology: Detailed testicular histopathology examination was conducted in order to identify treatment-related effects such as retained spermatides, missing germ cell layers or types, multinucleated giant cells or sloughing of spermatogenic cells into the lumen. Examination of the intact epididymis included the caput, corpus, and cauda. The epididymis was evaluated for leukocyte infiltration, change in prevalence of cell types, aberrant cell, and phagocytosis of sperm.

Quantitative Ovarian Histopathology: Ovarian histopathology, in addition to qualitative examination, included quantitative evaluation of primordial follicles, growing follicles and antral follicles and corpora lutea from 10 section per ovary for the first F1 females in groups 1 and 5. Additionally, corpora lutea were counted on one section per ovary.

8. Seminology and Spermatid Count

Sperm analysis was performed for the first 10 males per group (P generation). For the F1 parent generation sperm analysis was performed on all males per group.

Motility: At necropsy of adult males an epididymial sperm sample was obtained from the left caudal epididymis of each male. The sample was diluted with a pre-warmed physiological medium, and rapidly after obtained, one hundred sperm were counted

microscopically for determination of percentage of not motile, stationary motile and progressively motile sperm.

Morphology: A sperm sample from the caudal epididymidis was also used for morphological assessment after fixation and Eosin staining. Five hundred sperm per sample were evaluated microscopically. Morphological sperm evaluation was performed initially only for groups 1 and 5 males. In the absence of a treatment-related effect the slides for the group 2, 3 and 4 were not evaluated.

Sperm, Spermatid Count: The left caudal epididymis and left testis were taken for determination of homogenization-resistant spermatids and caudal epididymal sperm reserve. These tissues were frozen at -20 ± 5 °C pending evaluation. For evaluation the weighed tissues were placed in Triton-X-100 solution and homogenized with a blender (Ultra Turrax) and ultrasonic water bath. Sperm or spermatid heads were counted microscopically using a modified Neubauer chamber. These evaluations were performed in the first instance only for group 1 and 5 males. In the absence of treatment-related effect the remaining frozen tissue were not evaluated.

9. Data Compilation and Processing

Data for food consumption, body weights, reproduction and litter data, organ weights, macroscopic findings at necropsy and histopathology were recorded on-line (RCC-TOX LIMS) and used to calculate fertility indices, mean pre-coital time, pre- and post-implantation losses, mean litter size, pup sex ratios and viability indices. Group means were calculated according to the definition of any mean value using the individual values per animal and the number of animals.

10. Statistical Analysis

Univariate one-way analysis was used to assess the significance of intergroup differences. For variables assumed to follow a normal distribution, the Dunnett many-one t-test, based on a pooled variance estimate, was used for intergroup comparisons (i.e. single treatment groups against the control group). When data could not be assumed to follow a normal distribution, the Steel test (many-one rank test) was applied. Fisher's Exact test for 2x2 tables was applied if the variables could be dichotomized without loss of information.

II. RESULTS

DATA FOR P GENERATION ANIMALS

P Generation Male and Female Detailed Clinical Observations (Daily and Weekly)

No unscheduled deaths occurred in this study and no Flutriafol-related clinical signs or behavioral changes were noted in any dose group at any time during the study, (see Summary Tables on pages 123 and 127 of the study report). Incidental findings such as localized hair loss, crusts or wounds occurred in individual animals without a dose related pattern.

P Generation Food Consumption and Body Weights

Total and Relative Food Consumption - P Generation Males

70 day Preparing and After Pairing Periods: Mean food consumption in animals exposed to Flutriafol during the 70 day Preparing and the After Pairing periods was not different from controls in a dose responsive manner at any time during the exposure periods (Table 2) (Figures on pages 62 and 64 and Tables on pages 138 and 142 of the study report). Relative food consumption was also

not affected by treatment with Flutriafol. Differences in mean values were not dose dependent (see Figures on pages 63 and 65 and Tables on pages 141 and 144 of the study report).

TABLE 2. Summary results of food consumption (g/animal/day) of P males exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
P Generation Pre-Pairing Period (n=24)					
Day 1-8	21.6±1.8	20.1±1.4**	22.2±1.9	21.7±1.7	22.4±1.4
Days 15-22	22.9±1.7	22.8±1.5	24.0±1.5	23.0±1.3	23.7±1.8
Days 29-36	23.0±1.6	22.4±1.3	23.2±1.6	23.1±1.5	23.6±1.9
Days 43-50	22.2±1.8	21.8±1.4	23.5±1.7*	23.0±1.7	24.2±1.7**
Days 57-64	22.9±1.9	22.4±1.5	23.7±1.8	23.4±1.8	23.3±2.0
Days 64-70	22.3±1.4	22.2±1.4	22.7±1.6	22.6±1.8	22.3±1.9
P Generation After Pairing Period (n=24)					
Days 1-8	22.7±1.9	22.4±1.5	23.1±1.8	22.9±1.9	23.3±2.0
Days 8-15	21.7±1.9	22.2±1.7	22.5±1.8	23.0±1.8*	22.8±1.8
Days 15-22	23.2±1.9	22.3±1.6	22.5±2.3	23.0±1.9	22.9±2.2
Days 22-27	22.7±1.9	22.1±1.8	22.5±2.1	22.2±1.8	23.4±2.2

* = Dunnett-Test based on pooled variance significant at 5%

** = Dunnett-Test based on pooled variance significant at 1%

Data obtained from pages 138 and 142 in the study report

Body Weights and Body Weight Gains - P Generation Males

70 day Preparing and After Pairing Periods: Mean body weights were similar in all dose groups (0, 30, 80, 150 and 300 ppm) and controls during the Preparing and After Pairing periods. Mean body weight gains did not show treatment related effects (Tables 3 and 4). Fluctuations in mean values of both mean body weights and body weight gains were considered to reflect the normal range of biological variability (See Figure on page 66 and Summary Tables on pages 145 of the study report).

TABLE 3. Summary results of body weight (g) of P males exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
P Generation Pre-Pairing Period (n=24)					
Day 1	204±15.9	202±16.4	206±16.8	203±15.7	203±11.4
Days 22	308±20.7	305±19.0	310±20.5	308±21.7	306±18.5
Days 50	380±28.0	371±24.3	378±26.6	377±29.0	377±26.0
Days 70	412±32.5	401±30.0	410±29.6	410±33.4	407±28.1
P Generation After Pairing Period (n=24)					
Days 1	437±35.4	426±30.0	438±33.0	436±35.8	431±31.3
Days 8	440±34.6	431±30.1	444±33.6	440±37.8	435±30.5
Days 15	447±34.3	440±31.3	451±33.3	448±36.9	445±30.0
Days 22	451±34.1	444±30.8	455±35.3	454±39.2	451±29.9

Non significantly different from control

Data obtained from pages 145 and 149 in the study report

TABLE 4. Summary results of body weight gain (%) of P males exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
P Generation Pre-Pairing Period (n=24)					

Day 1	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Days 22	52±9.1	52±7.4	51±6.6	52±5.4	51±7.4
Days 50	87±13.5	85±11.9	84±9.9	86±9.2	86±11.6
Days 70	103±15.3	100±14.4	100±10.8	102±11.8	101±13.8
P Generation After Pairing Period (n=24)					
Days 1	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Days 8	1±1.3	1±0.8	1±1.1	1±1.2	1±1.1
Days 15	2±2.5	3±1.2	3±1.3	3±1.7	3±1.2
Days 22	3±3.1	4±1.2	4±1.6	4±2.0	5±2.0

Non significantly different from control

Data obtained from pages 146 and 150 in the study report

Total and Relative Food Consumption - P Generation Females

Preparing, Gestation and Lactation Periods: In P generation females, total and relative food consumption mean values between exposed groups and controls did not show dose dependent differences during either the Preparing Period, or the Gestation and Lactation periods (Table 5). Thus, there was no indication of Flutriafol-related effects (See Figures on pages 72 to 77 and Summary Tables on pages 154, 157, 158, 160, 161 and 163 of the study report).

TABLE 5. Summary results of food consumption (g/animal/day) of P females exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
P Generation Pre-Pairing Period (n=24)					
Day 1-8	16.0±1.1	15.0±1.0*	15.7±1.3	16.3±1.0	16.8±1.6
Days 22-29	17.3±1.4	17.0±1.0	16.7±1.4	17.4±1.3	17.6±1.6
Days 36-43	17.0±1.5	16.5±1.3	15.9±1.4*	17.1±1.7	16.9±1.8
Days 43-50	16.3±1.5	16.1±1.4	16.6±1.3	17.0±1.6	17.7±2.1**
Days 57-64	17.0±2.5	16.3±1.5	16.9±1.4	16.8±1.8	17.0±2.2
Days 64-70	16.4±1.6	15.9±1.6	15.8±1.6	16.4±1.7	16.4±2.0
P Generation Gestation Period (n=20-23)					
Days 0-7	19.7±2.1	19.4±1.7	19.0±2.2	19.5±1.8	20.0±1.8
Days 7-14	21.1±1.8	21.0±2.1	20.7±2.0	21.3±1.7	21.7±2.0
Days 14-21	23.3±1.9	22.7±2.1	22.7±1.7	22.1±1.8	22.4±2.0
P Generation Lactation Period (n=21-23)					
Days 1-7	34.7±5.5	32.9±5.5	34.2±6.5	34.6±3.0	33.5±5.1
Days 7-14	55.2±4.0	53.3±4.7	52.9±5.3	56.8±8.0	52.8±6.6

* = Dunnett-Test based on pooled variance significant at 5%

** = Dunnett-Test based on pooled variance significant at 1%

Data obtained from pages 154, 158 and 161 in the study report

Body Weights and Body Weight Gains - P Generation Females

Preparing, Gestation and Lactation Periods: Mean body weight and body weight gains were not affected by Flutriafol treatment at any of the dose levels used in this study (30, 80, 150 and 300 ppm) (Tables 6 and 7). Differences between means were not dose dependent and appeared to be incidental in nature (See Figure on page 78 and Summary Tables on page 164 of the study report).

TABLE 6. Summary results of body weight (g) of P females exposed to dietary doses of Flutriafol Technical

	Dose (ppm)
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Flutriafol / PC Code 128940

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OPPTS 870.3800, OECD 416

Time	0	30	80	150	300
P Generation Pre-Pairing Period (n=24)					
Day 1	161±11.5	160±11.0	155±13.4	158±9.0	157±9.1
Days 22	205±14.4	203±14.3	200±17.1	205±11.9	202±12.9
Days 50	233±15.0	231±14.7	226±18.5	233±14.5	228±15.3
Days 70	245±15.9	243±15.2	239±19.7	247±16.3	240±14.4
P Generation Gestation Period (n=20-23)					
Days 0	245±14.0	247±13.8	240±18.9	251±13.6	243±14.2
Days 7	268±15.8	268±15.2	261±20.5	271±14.9	264±15.0
Days 14	292±17.2	293±16.8	284±21.6	296±17.1	288±17.1
Days 21	362±19.6	362±21.5	352±28.4	359±25.2	355±21.7
P Generation Lactation Period (n=21-23)					
Day 1	266±15.8	266±15.3	258±21.3	262±14.9	259±18.3
Day 4	281±16.9	279±15.1	273±22.3	280±14.2	272±16.3
Day 7	290±16.5	288±17.1	281±23.1	289±14.8	283±16.1
Day 14	308±16.8	303±17.8	299±24.1	304±17.6	300±14.8
Day 21	283±22.0	279±21.4	278±26.2	287±24.6	286±18.8

Non significantly different from control

Data obtained from pages 164, 168 and 171 in the study report

TABLE 7 . Summary results of body weight gain (%) of P females exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
P Generation Pre-Pairing Period (n=24)					
Day 1	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Days 15	20±4.1	21±3.2	23±3.8*	24±3.6**	23±3.7
Days 50	45±5.3	44±6.3	46±7.4	48±7.3	45±6.2
Days 70	52±6.3	52±7.4	55±9.1	56±8.6	53±6.0
P Generation Gestation Period (n=20-23)					
Days 0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Days 7	9±1.8	8±2.1	9±2.0	8±1.8	9±2.3
Days 14	19±2.8	19±3.3	18±3.2	18±2.9	18±3.3
Days 21	48±4.3	47±5.8	47±6.9	43±5.9*	46±5.0
P Generation Lactation Period (n=21-23)					
Day 1	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Day 4	6±3.7	5±3.7	6±4.4	7±3.6	5±5.0
Day 7	9±3.7	8±5.1	9±5.3	11±4.1	9±4.9
Day 14	16±4.0	14±5.5	16±5.9	16±5.9	16±5.9
Day 21	6±8.0	5±8.3	8±8.8	10±9.1	11±8.6

* = Dunnett-Test based on pooled variance significant at 5%

** = Dunnett-Test based on pooled variance significant at 1%

Data obtained from pages 165, 169 and 172 in the study report

Test Item Intake Values

Based on food consumption data in males and females during different dosing periods the following tables (Table 8 and 9) provides the mean dose levels achieved for Flutriafol in this study.

TABLE 8: Flutriafol Technical Intake in P Generation Males*

Study Period	Generation	Concentration (ppm)	Mean Achieved Dose Level (mg/kg bw/day)
Prepairing	P	0	0

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		30	2.0
		80	5.5
		150	10.2
		300	20.8
After Pairing	P	0	0
		30	1.5
		80	4.0
		150	7.7
		300	15.6

Food intake data for this table were presented on pages 41 and 42 of the study report

TABLE 9: Flutriafol Technical Intake in P Generation Females*

Study Period	Generation	Concentration (ppm)	Mean Achieved Dose Level (mg/kg bw/day)
Prepairing	P	0	0
		30	2.3
		80	6.2
		150	11.6
		300	23.9
Gestation	P	0	0
		30	2.1
		80	5.6
		150	10.3
		300	21.4
Lactation	P	0	0
		30	4.4
		60	12.1
		240	23.2
		1000	44.9

Food intake data for this table were presented on pages 43 and 44 of the study report

Reproduction Data (P Generation)

Prepairing estrous cycles: There were dose dependent differences in the mean number of days from estrus to estrus in P generation females in any of the Flutriafol exposure groups during the Prepairing period. The values varied from 4.9 days in the controls to 4.2, 4.2, 5.1 and 4.0 days in the 30, 80, 150 and 300 ppm groups, respectively. Thus there was no evidence of a Flutriafol-related effect on the estous cycle of the P generation females (See Summary Table on page 178 of the study report).

Mating Performance and Fertility: All females mated, however not all females became pregnant after mating. As shown by the Fertility index and Conception rates in Table 10, dose related effects on these parameters were not seen and the differences between the exposed groups and the controls were not statistically significantly different (see Table pages 179 and 180) of the study report). Thus, there do not appear to be Flutriafol related effects on mating performance and fertility in the P generation females.

TABLE 10. Fertility Indices for P Generation Females

	Group 1 0 ppm	Group 2 30 ppm	Group 3 80 ppm	Group 4 150 ppm	Group 5 300 ppm

P Generation Females					
Percentage mating	100.0	100.0	100.0	100.0	100.0
Fertility index (%)	95.8	87.5	95.8	91.7	87.5
Conception rate (%)	95.8	87.5	95.8	91.7	87.5
Gestation Index (%) (Breeding)	100.0	100.0	100.0	100.0	100.0

Percentage mating = (Females mated / Females paired) x 100

Fertility index = (Females achieving a pregnancy / Females paired) x 100

Conception rate = (Females achieving a pregnancy / Females mated) x 100

Gestation index = (Number of Females with living pups / Number of females pregnant) x 100

#, ##: Fisher's Exact Test significant at 5% (#) or 1% (##) level

Data from page 120 of the study report

Breeding Data - P Generation:

The gestation index is calculated as the number of females with living pups divided by the number of pregnant females. As shown in Table 10, the gestation index was not negatively affected by Flutriafol treatment at any of the four doses used in this study (30, 80, 150 and 300 ppm). The Birth Index for the P Generation (the number of pups born alive/number of implantations) x 100 is shown in Table 11. There were no statistically significant differences between the control the four treated groups, however the birth index in the highest dose group (300 ppm) is lower than that the other groups.

Duration of gestation: The duration of gestation was similar in all groups and thus was not affected by Flutriafol at the doses used in this study (see Summary Table page 181 on Page 181 of the study report).

Implantations: The number of implantations per dam was similar in all dose groups and no dose response differences were observed. Thus there were no Flutriafol-related effects on the number of implantations (see Summary Table on page 181 of the study report).

Post Implantation Loss: There were no statistically significant decreases among the exposure groups and the controls for the three indices calculated for this outcome measure, however all three of the indices (% post implantations, total number of post implantations and mean number of post implantations) had lower mean values in the highest dose group (300 ppm) than the control group (Table 11).

Dead and Live pups at first litter check: The litter size at first litter check was similar among the control and treatment groups and there was no statistically significant difference between the mean number of dead pups in any of the groups. However, there was a large difference in the number of dead pups between the control and 300 ppm group (Table 11). At first litter check, 8 pups were found dead in the 300 ppm group compared to 1 pup in the control group. The Investigators state that the total number of affected litters was within the historical reference range for the laboratory. The total number of dead pups was outside the range of the historical F1 breeding reference data, but within the range of the corresponding data for F2 breeding. Based on all of these observations, the Investigators concluded that the higher number of dead pups observed was incidental rather than related to the Flutriafol exposure (see Summary Tables on pages 180 and 181 and Historical reference data on page 1559).

Post natal loss Days 0-4 post partum: Similar to pup loss observed at the first litter check, there was a non-significantly higher number of pups lost during Days 0-4 post partum in the

highest dose group (300 ppm) compared to the other four groups. Two pups were lost in the control, 30 and 80 ppm groups, one pup was lost in the 150 ppm group and 5 pups were lost in the 300 ppm group. The Investigators concluded that since the numbers of pups lost in each group was within the range of the historical reference data, these findings were considered to be incidental (See Summary Tables on pages 181 and 201 and Historical reference data on page 1559 of the study report).

Breeding Loss Days 5-21 post partum: One pup was lost in the 80 ppm dose group and 5 pups were lost in one litter in the 150 ppm dose group (total litter loss). (Summary Tables on page 181).

Living pups Day 21 post partum: Consistent with the observed effects of the 1000 ppm dose of Flutriafol on pup survival from first litter check through the lactation period, the number of living pups at day 21 post partum was lower in the 1000 ppm dose group compared to the control group and to the other Flutriafol dose groups.

TABLE 11. Breeding Data Per Group Calculated For P Generation Females.

Index %	Group 1 0 ppm	Group 2 30 ppm	Group 3 80 ppm	Group 4 150 ppm	Group 5 300 ppm
P Generation					
Birth Index	91.2	90.9	91.8	90.1	89.4
Viability Index	99.3	99.2	99.3	99.6	98.0
Weaning Index	100.0	99.4	100.0	97.1*	100.0
Post Implantation Loss					
% Implantations	8.8	9.1	8.2	9.9	10.6
Total Number	27	26	24	29	30
Mean + SD	1.2 + 1.19 ^a	1.2 + 1.14	1.0 + 1.52	1.3 + 1.17	1.4 + 1.54
Dead Pups at First Litter Check					
Total Number	1	0	0	1	8
Mean + SD	0.0 + 0.21	0.0 + 0.0	0.0 + 0.0	0.0 + 0.21	0.4 + 1.32
Post Natal Loss Day 0-4					
Total Number	2	2	2	1	5
Mean + S.D.	0.1 + 0.29	0.1 + 0.3	0.1 + 0.29	0.0 + 0.21	0.2 + 0.89
Total Litters Affected	3/23 (13%)	2/21 (9.5%)	1/23 (4%)	2/22 (9%)	4/21 (19%)
Total Loss of Pups	3	2	2	2	13
% Living Pups	1	0.7	0.7	0.7	4.9
Living Pups at Day 21 post partum					
% male/% females	53/48	49/51	50/50	50/50	50/50
Total Number	184	167	181	165	161
Mean + S.D.	8.0 + 0.0	8.0 + 0.22	7.9 + 0.34	7.5 + 1.79	7.7 + 1.32

#, ## Fisher's Exact Test significant at 5% (*) and 1% (##) level.

Birth Index = (number of pups born alive/number of implantations) x 100

Viability index = (number of alive pups on day 4 p.p./ number of pups born alive) x 100

Weaning index = (number of alive pups on day 21 p.p./number of alive pups on day 4 p.p.) x 100

^a Data presented as means ± standard deviation

Data from Tables on pages 181-182 the study report.

Sperm Analysis Data (P Generation)

Sperm Motility: There were no statistically significant differences in sperm motility measures between treatment groups (Table on page 184 of the study report).

Sperm Morphology: Five categories of sperm morphology were examined in the control (Group 1) and the 300 ppm (Group 5) groups only. There was no difference in the % sperm with normal hook and tail (83.3% versus 93.3% for Group 1 and Group 5, respectively). There was also no evidence of an effect of Flutriafol on sperm morphology when any of the five specific categories of misshapen sperm were examined (Table on page 116 of the study report).

Sperm Head Counts: Mio spermatids were counted in control and 300 ppm Flutriafol caudal epididymides and testes after homogenization of the tissues. There was no statistically significant difference between the number of mio spermatids per gram of tissue in the control versus the 300 ppm exposed group and all values were within normal values for the rat species and strain used in this study (Table on page 184 of the study report).

Organ Weight (P Generation)

Organ to body weight ratios for the liver were statistically significantly increased in the highest Flutriafol dose group (300 ppm) in both males and female rats (Table 12). This increase was accompanied by histological changes seen as slight increases in fatty deposits in both males and females, and as hepatocellular hypertrophy in males.

There were no differences in organ weight or organ to body weight ratios between the control and any of the four dose group for any other organs, including the testes (See Summary Table on page 187).

TABLE 12. Summary results of organ/body weight ratios of P generation exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
P Male (n=24)					
Body weight (g)	451.5±32.5	441.1±29.7	452.8±35.5	450.5±38.2	448.7±28.8
Liver (g)	12.12±1.26	11.40±1.76	11.89±1.54	12.15±1.39	12.82±1.27
Liver (%)	2.68±0.16	2.57±0.30	2.62±0.22	2.70±0.20	2.86±0.20*
Thyroid (L) (g)	0.001±0.004	0.012±0.002	0.012±0.003	0.012±0.004	0.012±0.002
Thyroid (L) (%)	0.002±0.001	0.003±0.001	0.003±0.001	0.003±0.001	0.003±0.001
P Female (n=21-23)					
Body weight (g)	283.1±16.7	279.1±16.1	279.0±21.1	285.9±15.2	285.0±14.4
Liver (g)	12.11±1.90	11.95±1.63	11.92±1.81	12.71±1.08	13.21±1.23
Liver (%)	4.27±0.58	4.27±0.47	4.26±0.49	4.44±0.23	4.64±0.38*
Thyroid (L) (g)	0.009±0.002	0.009±0.002	0.008±0.002	0.008±0.001	0.009±0.002
Thyroid (L) (%)	0.003±0.001	0.003±0.001	0.003±0.001	0.003±0.001	0.003±0.001

* = Dunnett-Test based on pooled variance significant at 5%

** = Dunnett-Test based on pooled variance significant at 1%

Data obtained from pages 187, 189, 193 and 194 in the study report

Macroscopic Findings at Necropsy (P Generation)

There were no macroscopic findings in P Generation rats that indicated an adverse effect of Flutriafol in any of the treatment levels (see Summary Table on page 196 of study report).

Microscopic Findings (P Generation)

Liver: Centrolubular hepatocellular hypertrophy was noted in 5 males in the 300 ppm group in the P generation (see Appendix V Pathologist's report on page 1080).

DATA FOR F1 PUPS**Clinical Signs or Observations at Birth (First Litter Check) and During Lactation and Weaning**

No Flutriafof-related abnormal findings were observed in the F1 pups at birth or during the lactation and weaning periods. All abnormalities observed were considered incidental as they were of various types and not dose dependent; thus they appeared to be isolated incidences (see Summary Tables on pages 199 and 200 in the study report).

F1 Sex Ratios

The sex ratios of the F1 pups in the control and four treatment groups did not appear to be altered due to exposure to Flutriafof. There were no dose dependent differences and the statistically significant difference between the ratio observed in the lowest treatment group (30 ppm) and the control group was considered to be of an incidental nature.

F1 Pup Weights at Weaning (Day 21 Post Partum)

Mean body weights in the F1 pups at Weaning (Day 21 post partum) and body weight gains during the Lactation period were similar in all dose groups. Thus, there did not appear to be an effect of Flutriafof exposure on body weight and body weight gains.

Sexual Maturation - F1 Pups

Males: Sexual maturation in male F1 pups, as measured by day of preputial separation, was similar between controls and the lower exposure groups (30, 80 and 150 ppm), while a statistically significant increase in time to preputial separation was seen in the highest treatment group (300 ppm) (Table 13). The Investigators concluded, however, that since the day of preputial separation observed in the 300 ppm group was within the range of the historical control data (range 26.7 to 28.3), the increase in the 300 ppm group was not considered to be Flutriafof-related (see Summary Tables on page 206 and the Historical reference data on page 1559 of the study report).

Females: The mean day on which vaginal patency occurred was the parameter used to measure sexual maturation in females. There were no statistically significant differences between any of the four Flutriafof treatment groups and the control group in the F1 pups and all mean values were within the range of the historical control data (range 33.0 – 35.1) (Table 13) (see Summary Tables on page 206 and the Historical reference data on page 1559 of the study report).

TABLE 13: Sexual Maturation Summary for F1 Males and F1 Females (mean + S.D.)

	Group 1 0 ppm	Group 2 30 ppm	Group 3 80 ppm	Group 4 150 ppm	Group 5 300 ppm
F1 Males (n=24)					
Days to Preputial Separation	27.1 + 0.8	27.2 + 0.9	27.5 + 1.3	27.6 + 0.9	27.9 + 0.9 *
Body Weight (g)	78.74 + 6.27	81.72 + 9.63	81.61 + 7.28	83.78 + 8.53	83.55 + 7.79
F1 Females (n=24)					
Days to Vaginal Patency	33.7 + 1.5	33.5 + 1.6	34.4 + 1.2	33.6 + 1.3	34.6 + 1.8

Body Weight (g)	108.11 + 9.12	109.49 + 10.40	110.93 + 10.60	110.47 + 7.83	112.89 + 10.99
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*, ** Dunnett-test based on pooled variance significant at 5% (*) or 1% (**) level

+ Steel-test significant at 5% level

Organ Weight Data for F1 Pups

The mean organ weights and organ to body weight ratios were similar in males and females across all dose groups (see Summary Table on pages 208).

Macroscopic Findings for F1 Pups

Abnormalities noted by macroscopic examination at the end of the F1 treatment period were similar amongst groups and none appeared to be related to Flutriafof exposure (see Summary Tables on page 214 of the study report).

Parent Animals – F1 Generation

Detailed Clinical Observations (Daily and Weekly): Clinical signs from daily and weekly observations that occurred in control and exposed mature F1 animals were not dose dependent and appeared to be isolated instances. Thus they were considered to be incidental and not related to Flutriafof exposure (see Summary Tables on page 216 and 220 of the study report)

Total and Relative Food Consumption – F1 Generation Males: During the Preparing Period, mean food consumption was similar in all dose groups (Table 14). In the After Pairing Period, only between Day 15-22 in the highest dose group (300 ppm), food consumption was statistically significantly increased. However, because this increase did not occur consistently throughout the after pairing period, it was considered to be an incidental finding (see Figures on pages 92 and 94 and Summary Tables on pages 232 and 238 of the study report).

Statistically significant differences in relative food consumption between dose groups and controls occurred for short periods of time during both the pre-airing and the after pairing periods in males. Relative food consumption was statistically significantly increased in the 300 ppm group during the first three weeks of the pre-pairing period. Between days 85-91, relative food consumption was lower in the 80, 150 and 300 ppm groups. In the After Pairing period, relative food consumption was statistically significantly increased in the 300 ppm group on days 15-22. Since there was no dose-dependency in any of these alterations, these instances were considered to be incidental and not related to the Flutriafof (See Figures on page 93 and 95 and Summary Tables on pages 236 and 240).

TABLE 14. Summary results of food consumption (g/animal/day) of F1 male exposed to dietary doses of Flutriafof Technical

Time	Dose (ppm)				
	0	30	80	150	300
F1 Generation - Pre-Pairing Period (n=24)					
Day 1-8	17.8±2.1	17.0±2.1	18.2±1.5	18.0±1.6	18.0±1.7
Days 15-22	22.6±1.6	22.3±1.4	22.9±1.5	22.9±2.0	23.0±1.0
Days 36-43	23.6±1.5	23.4±1.9	23.4±1.9	23.6±2.0	23.5±1.4
Days 57-64	23.4±1.8	23.1±2.0	23.2±2.2	23.8±2.1	23.3±1.5
Days 78-85	23.8±1.9	23.2±2.0	23.1±2.2	23.6±2.0	22.9±2.2
Days 85-91	25.0±1.8	23.9±2.1	24.0±2.2	24.2±1.9	24.0±1.9
F1 Generation After Pairing Period (n=24)					
Days 1-8	24.6±1.9	23.6±2.2	23.9±2.3	24.2±2.2	25.3±1.6

Days 8-15	22.9±1.9	22.4±1.9	22.4±2.1	23.2±2.0	23.7±1.7
Days 15-22	23.5±2.0	22.8±2.0	23.7±2.3	24.2±2.3	25.4±1.8**
Days 22-28	23.7±2.0	22.5±1.7	22.7±2.5	23.1±2.1	23.9±1.8

* = Dunnett-Test based on pooled variance significant at 5%

** = Dunnett-Test based on pooled variance significant at 1%

Data obtained from pages 232 and 238 in the study report

Body Weights and Body Weight Gains – F1 Generation Males: During both the pre-pairing and after pairing periods, similar mean body weights and body weight gains were observed in all treatment groups (Tables 15 and 16). Small differences were not dose dependent and thus were not considered to be related to Flutriafol exposure (see Figures on page 96 and Summary Table on page 241 of study report).

TABLE 15 . Summary results of body weight (g) of F1 male exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
F1 Generation - Pre-Pairing Period (n=24)					
Day 1	109±16.7	102±17.5	114±10.4	111±12.8	106±14.7
Days 22	246±20.7	237±20.9	253±18.9	250±19.4	241±19.4
Days 50	365±23.3	359±30.2	366±30.5	366±28.4	361±22.1
Days 71	406±27.3	401±32.7	408±35.6	412±33.7	404±25.8
Days 91	430±29.8	424±38.4	429±38.4	439±35.5	428±33.6
F1 Generation - After Pairing Period (n=24)					
Days 1	453±31.2	448±42.2	449±38.2	457±36.3	453±33.0
Days 8	462±33.1	454±42.7	457±39.1	464±38.0	463±33.4
Days 15	470±34.1	463±42.9	468±40.2	477±40.4	474±32.9
Days 22	479±35.5	472±44.6	475±41.9	484±41.2	481±32.8
Days 29	473±36.0	467±44.7	471±42.2	485±41.0	478±35.1

Non significantly different from control

Data obtained from pages 241, 242 and 247 in the study report

TABLE 16. Summary results of body weight gain (%) of F1 male exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
F1 Generation - Pre-Pairing Period (n=24)					
Day 1	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Days 22	127±23.5	138±33.9	123±13.8	126±19.8	130±20.6
Days 50	240±48.2	263±76.5	223±29.5	232±42.1	247±51.2
Days 71	279±59.4	307±91.7	260±35.7	274±54.2	290±63.4
Days 91	301±66.7	330±101	279±39.7	299±59.3	313±72.8
F1 Generation - After Pairing Period (n=24)					
Days 1	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Days 8	2.0±1.1	1.0±1.6*	2.0±0.8	2.0±0.7	2.0±1.1
Days 15	4.0±1.6	3.0±1.1	4.0±1.3	4.0±1.3	5.0±1.3
Days 22	6.0±1.8	5.0±1.2	6.0±2.1	6.0±1.5	6.0±1.5
Days 29	4.0±2.2	4.0±2.5	5.0±8.7	6.0±1.8	6.0±1.9

* = Dunnett-Test based on pooled variance significant at 5%

** = Dunnett-Test based on pooled variance significant at 1%

Data obtained from pages 243, 244 and 248 in the study report

Test Item Intake Values: Based on food consumption data in males during different dosing periods the following tables (Table 17) provides the mean dose levels achieved for Flutriafol in this study.

TABLE 17: Flutriafol Technical Intake in F1 Generation Males*

Study Period	Generation	Concentration (ppm)	Mean Achieved Dose Level (mg/kg bw/day)
Prepairing	F1	0	0
		30	2.2
		80	5.7
		150	10.8
		300	22.1
After Pairing	F1	0	0
		30	1.5
		80	4.0
		150	7.4
		300	15.6

* Food intake data for this table were presented on page 50 of the study report

Total and Relative and Food Consumption – F1 Generation Females: Mean food consumption during the pre-pairing, gestation and lactation periods was not affected by Flutriafol exposure (Table 18). Similar values were measured in all groups (see Figures 102, 104 and 106 and Summary Tables on pages 252, 258 and 261 in the study report).

Small changes in relative food consumption were similar in the control and exposure groups, except for the following isolated instances. A statistically significant increase was observed in the 300 ppm group at the start of the Pre-pairing period. An increase in the 300 ppm group also occurred in the middle of the gestation period. Because these increases were not consistent throughout the treatment period they were considered incidental and the Investigators concluded that there was not effect of Flutriafol on relative food consumption (see Figures on pages 103, 105 and 107 and Summary Tables on pages 256, 260 and 263).

TABLE 18. Summary results of food consumption (g/animal/day) of F1 female exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
F1 Generation - Pre-Pairing Period (n=24)					
Day 1-8	14.5±1.3	13.8±1.7	14.9±1.2	14.2±1.1	14.8±1.2
Days 22-29	16.6±1.3	16.7±1.2	16.6±1.3	16.8±1.3	16.9±1.2
Days 43-50	16.9±1.7	16.2±1.7	16.6±1.7	16.5±1.5	16.8±1.5
Days 64-71	16.8±1.7	17.0±2.0	16.8±2.1	17.1±2.4	16.9±1.6
Days 85-91	18.9±2.1	18.0±2.8	17.8±2.5	18.1±2.8	17.6±1.5
F1 Generation - Gestation Period (n=20-23)					
Days 0-7	17.9±2.5	17.5±2.1	17.7±2.7	17.4±2.5	18.5±4.2
Days 7-14	21.0±1.9	20.6±1.9	21.3±2.1	20.8±1.8	21.3±2.1
Days 14-21	23.6±1.7	22.7±2.0	22.7±2.1	22.0±2.3*	22.6±2.0
F1 Generation - Lactation Period (n=21-23)					
Days 1-7	38.9±5.1	35.2±5.1	36.6±5.3	34.7±7.7	37.2±3.0
Days 7-14	55.9±3.6	53.3±6.2	55.5±4.4	53.0±9.5	55.2±3.3

* = Dunnett-Test based on pooled variance significant at 5%

** = Dunnett-Test based on pooled variance significant at 1%

Data obtained from pages 252, 253, 258 and 261 in the study report

Body Weight and Body Weight Gains – F1 Generation Females: Mean body weight and body weight gains were not affected by exposure to Flutriafol (Tables 19 and 20). The small decrease in mean body weight of the 30 ppm group on Day 21 post partum was considered to be incidental. The small decreases in body weight gains in the last week of the gestation period in the 150 and 300 ppm groups were considered to be minimal and of biological significance (see Figure on page 108 and Summary Tables on page 264).

TABLE 19. Summary results of body weight (g) of F1 female exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
F1 Generation Pre-Pairing Period (n=24)					
Day 1	100±12.7	92±17.4	102±9.0	101±12.4	95±12.3
Days 22	175±14.2	170±13.9	177±11.6	176±12.1	171±11.5
Days 43	219±14.4	214±13.9	220±13.3	222±14.0	212±15.6
Days 64	243±16.0	236±14.7	238±15.2	243±16.2	238±17.7
Days 91	257±19.7	252±18.9	253±15.3	262±19.0	253±19.5
F1 Generation - Gestation Period (n=20-23)					
Days 0	263±21.2	256±18.5	257±15.4	259±18.6	252±19.4
Days 7	278±24.0	271±21.2	272±17.2	275±17.2	267±20.5
Days 14	304±23.4	295±21.5	299±20.2	299±17.5	291±22.3
Days 21	378±27.8	366±28.9	368±26.8	365±28.5	354±26.2
F1 Generation - Lactation Period (n=21-23)					
Day 1	278±24.6	268±18.5	271±23.7	274±20.6	264±20.1
Day 4	296±23.4	285±17.0	291±21.5	291±22.6	281±19.3
Day 7	303±21.5	293±19.5	299±23.7	298±19.9	290±18.7
Day 14	314±19.2	304±17.8	311±21.8	308±21.2	300±17.0
Day 21	296±20.3	277±23.4**	294±21.0	295±16.9	283±19.9

* = Dunnett-Test based on pooled variance significant at 5%

** = Dunnett-Test based on pooled variance significant at 1%

Data obtained from pages 264, 265, 270 and 273 in the study report

TABLE 20. Summary results of body weight gain (%) of F1 female exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
F1 Generation - Pre-Pairing Period (n=24)					
Day 1	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Days 22	78±14.5	90±27.8	74±10.6	77±17.4	83±20.7
Days 50	134±25.8	154±47.5	126±17.4	132±25.1	138±31.8
Days 71	148±30.2	170±52.0	138±20.0	147±27.7	157±38.0
Days 91	162±35.4	183±54.9	150±24.2	163±32.0	170±41.8
F1 Generation - Gestation Period (n=20-23)					
Days 0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Days 7	6±1.9	6±2.3	6±1.7	6±2.7	6±2.3
Days 14	16±1.8	15±2.7	16±2.8	15±3.3	15±3.2
Days 21	44±4.5	43±4.1	43±4.9	41±6.1	40±6.4
F1 Generation - Lactation Period (n=21-23)					
Day 1	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
Day 4	7±5.0	7±5.1	7±4.2	6±4.1	7±4.7
Day 7	9±6.0	10±5.7	10±4.8	9±4.7	10±4.8
Day 14	13±6.3	14±6.6	15±5.9	13±5.2	14±5.3

Day 21	7±6.9	4±9.4	8±6.0	8±7.5	7±7.7
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Non significantly different from control

Data obtained from pages 266, 267, 271 and 274 in the study report

Test Item Intake Values: Based on food consumption data in females during different dosing periods the following tables (Table 21) provides the mean dose levels achieved for Flutriafol in this study.

TABLE 21: Flutriafol Technical Intake in F1 Generation Females*

Study Period	Generation	Concentration (ppm)	Mean Achieved Dose Level (mg/kg bw/day)
Prepairing	F1	0	0
		20	2.4
		60	6.3
		150	11.8
		300	24.5
Gestation	F1	0	0
		30	2.0
		80	5.3
		150	9.7
		300	20.7
Lactation	F1	0	0
		30	4.5
		60	12.2
		240	21.8
		1000	47.5

* Food intake data for this table were presented on page 52 of the study report

Reproduction Data – F1 Generation

Estrous Cycles in F1 Females: Estrous cycles were not affected by exposure to Flutriafol. The mean number of days from estrous to estrous was 4.3 in the control and 80 ppm groups, and 4.1 in the 30, 150 and 300 ppm groups (see Summary Tables on page 283 of the study report).

Mating performance and Fertility: Mating performance indices were not statistically significantly between the Flutriafol treated groups and controls, however fertility index and conception rate were lower in the 300 ppm group (Table 22). One female in the control group did not mate in the first pairing period, but mated when the male was replaced for a second pairing period. The date of the first mating of one female in the 150 ppm group and one in the 300 ppm group was missed.

One female in each of the control, 30 ppm, 80 ppm and 150 ppm groups was not pregnant. In the 300 ppm group, 3 females were not pregnant. All of the pregnant females, with the exception of one female in the control group that had only implantations, gave birth and raised their pups to day 21 post partum.

TABLE 22. Fertility Indices for F1 Generation Females

Indices %	Group 1 0 ppm	Group 2 30 ppm	Group 3 80 ppm	Group 4 150 ppm	Group 5 300 ppm
F1 Generation Females					
Percentage mating	100.0	100.0	100.0	100.0	100.0
Fertility index (%)	95.8	95.8	95.8	95.8	87.5
Conception rate (%)	95.8	95.8	95.8	95.8	87.5
Gestation Index (%) (Breeding)	95.7	100.0	100.0	100.0	100.0

Percentage mating = (Females mated / Females paired) x 100

Fertility index = (Females achieving a pregnancy/Females paired) x 100

Conception rate = (Females achieving a pregnancy / Females mated) x 100

Gestation index = (Number of Females with living pups / Number of females pregnant) x 100

#, ##: Fisher's Exact Test significant at 5% (#) or 1% (##) level

Data from page 285 of study report.

Duration of Gestation: The mean duration of gestation was similar in all dose groups.

Mean duration of gestation was 21.6, 21.7, 21.7, 21.5 and 21.6 days in groups 1, 2, 3, 4, and 5 respectively. Thus, there was no Flutriafol effect on duration of gestation (see Summary Table on page 286 of the study report).

Implantation Rate and Post Implantation Loss: The mean number of implantations per dam was not affected by treatment with Flutriafol. Similar numbers of implantations were seen in controls and all of the exposure groups.

None of the post implantation loss parameters were statistically significantly different between controls or any of the exposed groups, however there is a dose related increase in the % implantations, the total number of post implantations lost and the mean post-implantation loss per dam in the 150 and 300 ppm dose groups (Table 23).

Dead Pups at First Litter Check: The mean number of dead pups at first litter check were higher in the 150 and 300 ppm exposure groups compared to the control group. One pup was found dead at the first litter check in the control and 80 ppm groups, three pups were found dead from one litter in the 150 ppm group and 4 pups from one litter in the 300 ppm (Table 23). Although this is a dose dependent increase in dead pups, the Investigators concluded that because these were isolated occurrences in one litter in each group and because they were within the range of the historical reference data, they considered this finding to be incidental (see Summary Tables on pages 285 and 286 and Historical Reference Data on page 1578).

Post natal Loss – Day 0-4 Post Partum: There was a small increase in post natal loss in the 150 ppm group however this was not dose-related and was considered to be incidental to exposure to Flutriafol (Table 23) (See Summary Tables on page 286 and 306).

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Breeding Loss – Days 5-21 Post Partum: Only one pup was lost during Days 5-12 post partum. Loss of this pup was from the 150 ppm group and did not appear to be related to Flutriafol exposure.

TABLE 23. Breeding Data Per Group Calculated For F1 Generation Females.

Indices %	Group 1 0 ppm	Group 2 30 ppm	Group 3 80 ppm	Group 4 150 ppm	Group 5 300 ppm
F1 Generation					
Birth Index	91.7	92.5	91.7	90.7	89.3
Viability Index	99.6	98.9	99.0	97.8	99.2
Weaning Index	100.0	100.0	100.0	99.4	100.0
Post Implantation Loss					
% Implantations	8.3	7.5	8.3	9.3	10.7
Total Number	25	23	26	28	30
(mean + S.D.)	1.1 + 1.04	1.0 + 1.00	1.1 + 1.25	1.2 + 1.65	1.4 + 1.69
Dead Pups at First Litter Check					
Total Number	1	0	1	3	4
Mean + S.D.	0.0 + 0.21	0.0 + 0.0	0.0 + 0.12	0.1 + 0.63	0.2 + 0.87
Post Natal Loss Day 0-4					
Total Number	1	3	3	6	2
Mean + S.D.	0.0 + 0.21	0.1 + 0.46	0.1 + 0.34	0.3 + 0.69	0.1 + 0.44
Total Litters Affected	2/22 (9%)	2/23 (8.6%)	4/23 (17%)	4/23 (17%)	2/21 (9%)
Total Loss of Pups	2	3	4	9	6
% Living Pups	0.7	1.06	1.39	3.27	2.36
Living Pups at Day 21 post partum					
% male/% females	49/52	47/53	51/49	52/48	51/49
Total Number	176	180	183	170	166
Mean + S.D.	8.0 + 0.0	7.8 + 0.83	8.0 + 0.21	7.4 + 1.75	7.9 + 0.44

Fisher's Exact Test significant at 5% (*) and 1% (**) level.

Birth Index = (number of pups born alive/number of implantations) x 100

Viability index = (number of alive pups on day 4 p.p./ number of pups born alive) x 100

Weaning index = (number of alive pups on day 21 p.p./number of alive pups on day 4 p.p.) x 100

^a Data presented as means ± standard deviation per dam

Data from Tables on pages 286-287 of the study report.

Terminal Findings – F1 Parent Generation

Sperm Analysis Data

Sperm motility: Sperm motility was not affected by exposure to Flutriafol. Motility scores for the four exposure groups (30, 80, 150 and 300 ppm) were similar to those of the control group.

Sperm morphology: Sperm morphology was not affected by exposure to Flutriafol. The percentage of sperm with normal hood and tail was 90.6% in the 300 ppm exposure group versus 91.6% in the control group.

Sperm Head Count: Sperm head count numbers were within normal values for the rat species and strain used in this study. Mio spermatids concentrations in homogenized epididymal tissue were 707.87 Mio spermatids/gram in controls and 708.72 Mio spermatids/gram in the 300 ppm group. In testicular tissue the control group concentration was 136.37 Mio spermatids/gram in controls versus 136.37 Miospermatids/gram in the 300 ppm group (see Summary Tables on page 289 of the study report).

Organ Weight Data - F1 Generation

All mean organ weights and organ to body weight ratios were similar in males and females in all dose groups and controls except for the thyroid in males (Table 24). Statistically significant differences were observed in the 80 and 150 dose groups compared to controls, however these differences were small and were not dose dependent; thus they were considered incidental in nature (see Summary Tables on page 292 of study report).

TABLE 24. Summary results of organ/body weight ratios of F1 generation exposed to dietary doses of Flutriafol Technical

Time	Dose (ppm)				
	0	30	80	150	300
F1 Male (n=24)					
Body weight (g)	470.9±35.1	464.7±42.6	468±42.3	476±40.9	477±33.6
Liver (g)	12.78±1.37	12.29±1.68	12.65±2.07	13.48±1.54	13.61±1.22
Liver (%)	2.71±0.21	2.64±0.21	2.69±0.27	2.83±0.22	2.85±0.23
Thyroid (L) (g)	0.010±0.002	0.010±0.002	0.011±0.003	0.012±0.002**	0.010±0.002
Thyroid (L) (%)	0.002±0.00	0.002±0.00	0.002±0.001*	0.003±0.001**	0.002±0.00
F1 Female (n=21-23)					
Body weight (g)	293.6±22.8	277.9±18.1*	294.4±22.4	291.9±22.9	287.1±19.7
Liver (g)	13.81±3.37	12.23±2.38	13.49±2.13	13.40±1.84	13.69±1.80
Liver (%)	4.69±0.99	4.38±0.70	4.56±0.48	4.59±0.48	4.76±0.45
Thyroid (L) (g)	0.009±0.002	0.009±0.002	0.009±0.002	0.009±0.002	0.010±0.003
Thyroid (L) (%)	0.003±0.001	0.003±0.001	0.003±0.001	0.003±0.001	0.003±0.001

* = Dunnett-Test based on pooled variance significant at 5%

** = Dunnett-Test based on pooled variance significant at 1%

Data obtained from pages 292, 294, 298 and 299 in the study report

Macroscopic Findings - F1 Generation

No abnormal macroscopic findings were noted with a dose relationship to Flutriafol exposure in any of the F1 Parent Generation exposure groups. (See Summary Tables on page 301 of the study report).

Microscopic Findings - F1 Generation

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Liver: Centrilobular hepatocellular hypertrophy was noted in 9 males and 1 female in the 300 ppm group in the F1 generation (see Appendix V Pathologist's report on page 1080).

Sperm Staging: No Flutriafol related effects were noted. Abnormalities noted in this measure were not different between treated rats and controls. They are commonly noted in male rats of this strain and age.

Ovary Staging in F1 Females: The differential follicle count did not reveal any Flutriafol related effects

Data for F2 Pups

Clinical Signs or Observations at Birth (First Litter Check) and During Lactation to Weaning

No abnormal findings were noted at first litter check or during the lactation period which were considered to be related to Flutriafol exposure. The few observations were unrelated and of sporadic nature (see Summary Tables on pages 304 and 305 of the study report).

Sex Ratios

The sex ratios (% males/ % females) were not affected by exposure to Flutriafol. Mean sex ratios were 48/52, 47/53, 51/49, 52/48 and 51/49 in the controls, 30, 80, 150 and 300 ppm groups, respectively (see Summary Tables on page 286 of the study report).

Microscopic Findings

No pathological lesions were noted in the pups of the F1 or F2 generations (see Appendix V Pathologist's report on page 1080).

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

The feeding levels 30 ppm, 80 ppm, 150 ppm and 300 ppm corresponded to mean dose levels of 1.5-2.2, 4.0-5.7, 7.4-10.8 and 15.6-22.1 mg/kg body weight in the male groups and of 2.0-4.5-, 5.3-12.2, 9.7-23.2 and 20.7-47.5 mg/kg body weight in the female groups.

At 300 ppm, effects of the test item included:

Centrilobular hepatocellular hypertrophy in the P and F1 parental generation. These findings correlated with increased liver to body weight ratios in the P parental generation. The changes are indicative of an adaptive change to metabolic activation by a xenobiotic and not a toxic response to the administration of Flutriafol Technical.

At 150 ppm, 80 ppm and 30 ppm, no test item-related were noted.

Based on these results, the NOAEL (No Observed Adverse Effect Level) is 300 ppm for toxicity and the NOEL (No Observed Effect Level) for reproductive function. The NOEL for toxicity is 150 ppm.

B. REVIEWER'S COMMENTS:

RELIABILITY RATING: Acceptable/Guideline

OECD 416 Two-Generation Reproduction Toxicity Study
EPA OPPTS 870.3800 Reproduction and Fertility Effects

In P and F1 parental animals, no Flutriafol Technical-related clinical signs or observations were noted in this study. Mean and relative food consumption, body weights and body weight gains were also not affected by exposure to Flutriafol. The mean Flutriafol exposure dose levels achieved in the 300 ppm exposure group in this study were 20.8 mg/kg bw/day for P generation males, 22.1 mg/kg bw/day for F1 males, 23.9 mg/kg bw/day for P generation females and 24.5 mg/kg bw/day for the F1 generation females during the pre-pairing period. During gestation and lactation, P generation females in the 300 ppm group ingested 21.4 mg/kg bw/day and 44.9 mg/kg bw/day, respectively, while the F1 generation females in the 300 ppm group ingested 20.7 mg/kg bw/day and 47.5 mg/kg bw/day of Flutriafol. There were no Flutriafol-related abnormal findings in the F1 animals after weaning. Food consumption and relative food consumption were consistently non-statistically significant nor were they different from controls in a dose related manner. Small differences observed in body weight and body weight gain were also considered not related to Flutriafol treatment. Liver to body weight ratios were statistically significantly increased in both males and females in the 300 ppm group. There were no differences in any other organ weights or organ to body weight ratios between the control and Flutriafol exposed animals at any level of exposure. There were no dose dependent abnormal macroscopic findings at necropsy that were Flutriafol related. Histopathology of livers showed slight increases in fatty deposits in both sexes and evidence of centrilobular hepatocellular hypertrophy in males in the 300 ppm group, which is considered an adaptive change.

The Parental LOAEL is 300 ppm (18.2/22.6 mg/kg bw/day [M/F]) based upon increased relative liver weights, fatty deposits in the liver in both sexes as well as hepatocellular hypertrophy in males. The NOAEL is 150 ppm (9/10.8 mg/kg bw/day[M/F]).

An increased incidence of pup mortality was observed in the F1 and F2 pups at first litter check and 0-4 postnatal days. Mean and relative organ weights were not affected by Flutriafol exposure. Abnormalities noted by macroscopic examination showed no dose response relationship with Flutriafol treatment levels. No pathological lesions were noted in the F2 pups.

The offspring LOAEL is 300 ppm (18.5/22.6 mg/kg bw/day [M/F]) based upon increased pup mortality in the F1 pups at the first litter check and 0-4 postnatal days. The offspring NOAEL is 150 ppm (9/10.8 mg/kg bw/day [M/F]).

There were no significant Flutriafol-related effects on the length of estrous cycle, mating performance and fertility in the P generation female rats. Sperm analysis data indicated no effects of Flutriafol on sperm motility or morphology at any of the doses used in this study. There were also no differences between the sperm head counts per gram of tissue in testes and caudal epididymus. The duration of gestation and the gestation index were also not affected by Flutriafol exposure. Breeding data for the P generation females show no statistically significant differences in the birth and viability indices. The weaning index for the 150 ppm group was statistically decreased compared to controls. Since the values in the 300 ppm group were not lower than controls, this difference was considered an incidental finding. There were also no statistically significant dose related effects in all other breeding indices. Sexual maturation in F1 females (days to vaginal patency) was not altered by Flutriafol treatment. In males, sexual maturation (days to preputial separation) showed a slight delay (statistically significant, yet within historical control data) in the 300 ppm compared to controls. There were no Flutriafol-related effects on estrous cycles and gestation index in the F1 animals. Fertility index and conception rates were not statistically significantly different between exposure groups and controls, however small, non-statistically significant decreases were evident in the highest (300 ppm) group. Duration of gestation, viability index and weaning index were not altered by Flutriafol treatment. Birth index for the 300 ppm was not statistically significantly different from controls, however it was lower than all the other groups. There were also no statistically significant differences in the breeding parameter values of the dose groups compared to controls, however the post-implantation loss measures were highest in the 300 ppm group as were the number of dead pups per litter. Mean organ weights and organ to body weight ratios were similar in F1 males and females in all the dose

groups including controls and small changes were no related to Flutriafof dose. No Flutriafof-related effects were noted in sperm staging in males or differential follicle counts in females.

The reproductive LOAEL was not observed. The reproductive NOAEL is 300 ppm (18.2/22.6 mg/kg bw/day [M/F]).

This study is classified as **acceptable/guideline** for establishing a NOAEL for reproductive and fertility effects. It has met the requirements of the OPPTS 870.3800 and OECD 416 guidelines.

The guideline requirement that dose levels should be chosen to induce some reproductive and/or systemic toxicity at the high dose with the intermediate dose producing minimal observable toxic or reproductive effects was met if the results of the Gerspach (2008; MRID# 481969-21) preliminary study is considered together with this current study (MRID# 481969-22), giving a dose range from 30 to 1000 ppm Flutriafof.

C. DEFICIENCIES

Deviations:

1. The body weight range of the P generation animals at treatment start exceeded the target range stated in the study plan.
2. The diet formulation for group 2 (30 ppm) prepared on March 20, 2008, was approved for use by the study director, because the content of the pellets was within the range of acceptance, in spite of the analytical value of the powder being out of the range of acceptance.
3. The date of mating of one P generation female in group 1 and one female in group 5 was missed (Noted on page 44 of study report).

These deviations did not significantly affect the outcomes of this study.

D. REFERENCES

1. Gerspach, R. (2008). Flutriafof Technical: Preliminary Reproduction Toxicity Study in the Han Wistar Rat. Harlan Laboratories Ltd (Former Rcc Ltd), Wolferstrasse 4, CH-4414 Fullinsdorf, Switzerland. Harlan Laboratories Study Number A52705. MRID # 481969-21.